IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of

Applicant MASCAGNI Paolo et al.
Serial No. 09/763,581
Filed February 22, 2001
Title COMPLEXES OF PAROXETINE, WITH CYCLODEXTRINS OR CYCLO DEXTRIN DERIVATIVES
Examiner Leigh C. Maier
Art Unit 1623

DECLARATION UNDER CFR 1.132

I, Paolo Mascagni, being duly sworn depose and say that:

1. I am an Italian citizen residing in Villasanta (Milan, Italy).
2. I am familiar with the English language.
3. further declare that:

A) Universities or Colleges attended

I graduated in Chemistry in 1978 at the University of Florence, where completed a Post-Doctoral Research on cyclo-addition reactions in 1980.

B) Research activity

From 1980 until 1983 worked as a Postdoctoral Research Fellow at the Department of Biochemistry of the University of Wisconsin-Madison, WI, USA.

From 1983 (Jan) to 1984 (Dec) I worked as a Senior Research Associate at the University of London, School of Pharmacy, Department of Pharmaceutical Chemistry, London, UK.

My research activity was mainly devoted to the following research topics:

- One- and two-dimensional NMR spectroscopy;
- Synthesis of novel biopolymers for drug delivery;
- Synthesis and structure determination of phytotoxic peptides from fungi;

- Chemical synthesis of peptides and proteins, in particular HIV-1 peptides and proteins and bacterial heat-shock proteins;
- Development of peptide/protein synthesis and purification protocols;
- Structural studies of biopolymers and natural products (plant alkaloids, lipolysaccharides and peptides) using CD, FT-IR and NMR spectroscopies and computer modeling.

C) Publications

I am author or co-author of about 120 scientific publications in important International Journals and I am designated inventor of more than 20 patent applications, several of which granted world-wide.

D) Professional experiences

From 1985 (Jan) until 1988 (Dec) I worked as Lecturer at the School of Pharmacy, Department of Pharmaceutical Chemistry of the University of London, London, UK. In 1987 I was Host Professor at the California Institute of Technology, Pasadena, CA, USA.

In January 1989 I became Senior Lecturer of the above said School of Pharmacy of the University of London, and Visiting Professor at the Chemistry Department of the University of Florence, Italy.

My teaching activity at the University of London lasted until December 1990, and concerned courses to 1st, 2nd and 3rd year students in Organic Chemistry, Analytical Chemistry, Structural Analysis and Laboratory Practice.

From 1991 until today I have been worked for Italfarmaco S.p.A. in Milan, Italy, as Head of Peptide Chemistry and Computational Chemistry (1991-1994), as Head of Medicinal Chemistry (1994-1995), and Head of Pre-Clinical Research (1995-present).

In 2002 and 2003 I was Visiting Professor at the Faculty of Pharmacy of the University of Ferrara, Italy.

4 further declare that the following experiments were carried out under my direct responsibility.

a) The following experiment has been carried out in order to demonstrate that Ronsen et al. does not anticipate the present products as instantly claimed in claim 29.

In particular, the preparation of Example 5 of Ronsen et al. has been repeated by using β-cyclodextrin instead of hydroxypropyl-β-cyclodextrin. Furthermore increasing concentrations of β-cyclodextrin were used up to a paroxetine/ β-cyclodextrin ratio of

about 1 as one would expect in a 1:1 inclusion complex. In the following example the experiment referring to such a ratio is described:

2 g of amorphous paroxetine hydrochloride (5.5x10-3 moles), prepared as described in Example 1 of Ronsen et al., was dissolved in 150 ml of absolute ethanol. To the thus obtained solution 7 g of β-cyclodextrin (6.1x10-3 moles) in powder was then added, and the suspension kept under vigorous stirring. After 24 hours, the β-cyclodextrin was still in suspension, in accordance with the insolubility of non derivatised β-cyclodextrin in ethanol. The suspension was filtered and the solid thus collected extensively dried under vacuum. 6.8 g of solid was thus obtained which was shown to be β-cyclodextrin by DSC. No formation of the complex between paroxetine and cyclodextrin was thus observed, but practically all of the β-cyclodextrin (97%) used remained un-dissolved. Furthermore, upon evaporation under vacuum of the ethanol solution afforded paroxetine with no detectable traces of β-cyclodextrin.

From the above described experiment it emerges therefore that the present inclusion complexes between paroxetine and a cyclodextrin as claimed in claim 29 could not be prepared by the method disclosed by Ronsen et al.

b) The preparation already disclosed in Example 1 of the original application has been repeated as follows:

1 g of paroxetine base is dispersed in 150 g of deionized water under stirring. A solution of 0.11 g of HCl in 28 g of water is added to the dispersion under stirring. Stirring is continued until paroxetine is completely solubilised, then 3.5 g of β-cyclodextrin in powder are added to the solution and the obtained dispersion is heated to 40 °C under nitrogen flux and vigorous stirring for 3 hours. An opalescent solution is thus obtained containing a little amount of undissolved residue which is removed by filtration through a cellulose acetate filter having 0.45 μm porosity.

The obtained solution is freeze-dried and 4.3 g of a product with a molar ratio between paroxetine HCl and β-cyclodextrin of 1:1 and with a water content of 5.4% by weight is obtained. This product has been characterized by ^{1}H-NMR and Differential Thermal Analysis (DSC), then comparing the results with the results of the same analysis carried out on non-complexed paroxetine and β-cyclodextrine.

By means of ^{1}H-NMR 200 MHz using D_2O as the solvent, a spectrum was obtained showing a positive variation of the chemical shift for many protons of paroxetine in comparison with the spectrum obtainable for the paroxetine alone, whereas a negative variation is observed for the protons of β-cyclodextrin inside its cavity in comparison with the spectrum of the β-cyclodextrin. The chemical shift values were wholly homogeneous with those reported in Tables 2 and 3, pages 12 and 13 of the present application as filed, and proved that the product obtained as above described consists of a complex of paroxetine with β-cyclodextrin.

On this product a DSC test has been also carried out by using a Perkin-Elmer DSC7 equipment in a temperature range comprised between 50 and 300°C and with a heating range of 10°C/minute. The so obtained thermogram was homogeneous with that reported in the original application in Figure 2; in particular, a peak corresponding to the decomposition of the product was observed between 230 and 250°C, that is different from the decomposition peak observable for non-complexed paroxetine which is comprised between 260 and 300°C. This confirmed therefore the occurred complexation of paroxetine with the cyclodextrin.

c) The preparation disclosed in Example 8 of the original application was repeated in order to confirm that a complex between paroxetine and a cyclodextrin derivative, free from organic solvents and in particular free from ethanol, has been obtained by the Applicant.

1 g of paroxetine base is dispersed in 50 g of deionized water under stirring. A solution of 0.11 g of HCl in 28 g of water is added to the dispersion under stirring. Stirring is continued until paroxetine is completely solubilised, then 4.0 g of hydroxylpropyl β-cyclodextrin in powder is added to the solution and the obtained dispersion is heated to 40 °C under nitrogen flux and vigorous stirring for 3 hours. An opalescent solution is thus obtained containing a little amount of undissolved residue which is removed by filtration through a cellulose acetate filter having 0.45 μm porosity.

The obtained solution was freeze-dried and 4.7 g of a product were obtained. This product has been characterized by ^{1}H-NMR and Differential Thermal Analysis (DSC), in the same conditions disclosed above for experiment b), with the only exception that the DSC test been carried out in a temperature range comprised between 50 and 200°C The

formation of a complex was confirmed both from ^{1}H-NMR and DSC analysis.

5. further declare that all the statements of my own knowledge are true and that all the statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statement and the like so make are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Applicant or of any patent issuing thereon.

Cinisello Balsamo (Milan), 2004-09-02

Paolo Mascagni